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BIOLOGY AND DAMAGE OF *THAMNURGUS PEGANI* EGGERS (COLEOPTERA: SCOLYTIDAE) FEEDING ON *PEGANUM HARMALA* L. IN EASTERN TURKEY

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Abstract.—The scolyted beetle, *Thamnurgus pegani* Eggers, was recorded from eastern Turkey in 2002 on *Peganum harmala* L. (Zygophyllaceae), a perennial weed toxic to domestic animals. The beetle's developmental stages, biology, damage, and parasitoids were studied in Igdir Province and in the laboratory at Erzurum during 2003–2005. *Thamnurgus pegani* is univoltine and adults hibernate in the larval galleries, root crown, and soil beneath the host plant. Overwintering beetles appear at the end of April and move to the newly developed stems. Females oviposit eggs singly in the small holes, usually between the stem and lateral shoot. The emergent larvae tunnel downward in the stem pith. A fungus, *Fusarium oxysporium* Schlechtend was detected within the gallery and on the body of *T. pegani*. The fungal-infested tissue became blackishbrown. Larva feed on this infected tissue, pupate in the mined stems, and new adults appear during the first week of August. *T. pegani* attack on *P. harmala* reduces seed crop size and germinability, and thus the beetle is considered to be a potential candidate for biological control of the plant in areas of the world where it has been inadvertently introduced.

Key Words: *Thamnurgus pegani*, *Peganum harmala*, African rue, biology, biocontrol

The scolytid beetle, *Thamnurgus pegani* Eggers, was first detected on Syrian rue, *Peganum harmala* L. (Zygophyllaceae), 12 km south of the city of Igdir in the foothills of Mt. Ararat in eastern Turkey at an elevation of 1250 m (39° 47′ 46 N; 44° 36′ 44 E) in the summer of 2002 (Fig. 1). This site is towards east 302 km from Erzurum.

Schedl (1981) recorded about 40 species in the genus *Thamnurgus* from Europe, Palearctic Asia, Africa, and Madagascar. Species of *Thamnurgus* feed in the stems of various plant species in the genera of *Euphorbia*, *Peganum*, *Delphinium*, *Tamarix*, and *Labiatae* (Balachowsky 1949, Schedl 1981, Pfeffer 1995).

Peganum harmala L. (African rue) is an erect, 30–70 cm tall, perennial herbaceous perennial with stout stems arising from a woody rootstock. The leaves are alternate, with minute deciduous stipules. The flowers are solitary, usually with opposed leaves (Davis 1967). The flowering period is from March to April. The fruits are globose, three-chambered capsules that contain blackish, angular seeds (Davis 1967, Mahmoudian et al. 2002).

Peganum harmala L. is distributed throughout Turkey, below 1500 m except in the Black Sea Region (Davis 1967). However, it is more common in theeastern and central parts of Turkey

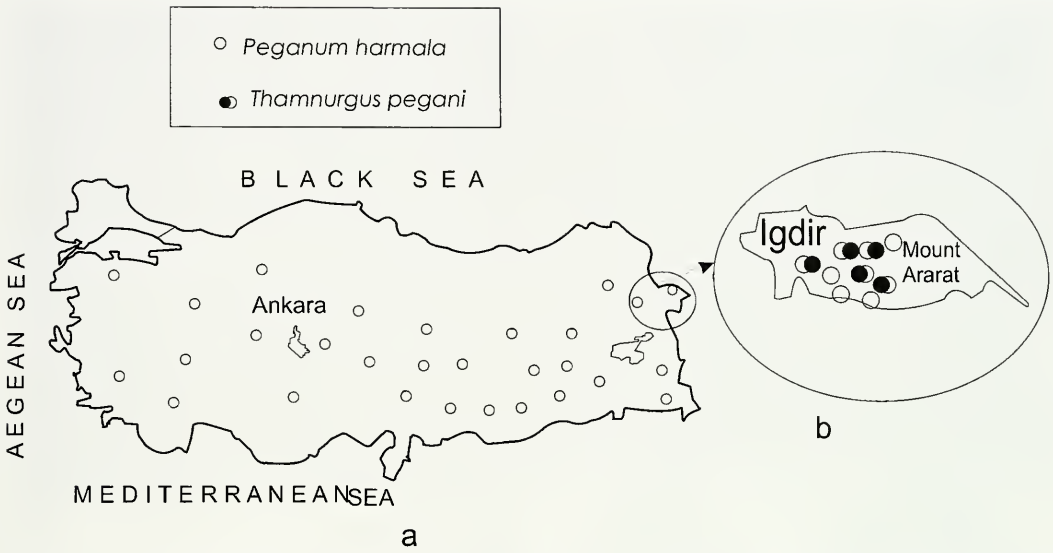

Fig. 1. a) Distribution of *Peganum harmala* in Turkey, b) *Thamnurgus pegani* occurrence in Igdir Province.

(Fig. 1). It is regularly encountered on the outskirts of some villages in the steppe area of eastern Anatolia. The plant has become problematic following its accidental introduction into the western USA.

Peganum harmala L. is known as 'uzerlik' or 'sedefotu' in Turkish. In villages the fruits of this plant have been used as adornment in the rooms of homes, especially by young women. They believe that these adornments protect the residence occupants against evil eyes. The plant traditionally has been used as an emmenagogue and an abortifacient in the Middle East and North Africa. All parts of the plant contain alkaloids that are toxic upon ingestion and severe intoxication occurs in domestic animals. Animals initially become prostrate and then anorexia, hypersalivation, vomiting and diarrhea occur (Bailey 1986, Bailey and Damn 1981, El-Bahri and Chemli 1991, Mahmoudian et al. 2002, and references therein). The plant usually is not grazed because of its bitter taste. However, when favored forage is sparse, animals may be attracted to and graze intermittently on *P. harmala* (Mahmoudian et al. 2002, and references therein).

Another *Thamnurgus* species, *T. euphorbiae* Küster, was recorded from *Euphorbia characias* L. in Italy, and research was conducted on its host relationships and behavior to determine its potential as a biological control agent for leafy spurge, *Euphorbia esula* L., an invasive weed, which causes over $ 100 million in economic losses in the USA (Anonymous 1992). Christofaro et al. (2000) studied its potential host range at the USDA-ARS-EBCL substation in Rome. Subsequent field and laboratory studies were conducted on the beetle's biology in Italy. Finally, *T euphorbiae* was approved by the Tecnichal Advisory Group for Biological Control Agents of Weeds for release as a bioagent against leafy spurge in the USA (Campobasso et al. 2004).

In this paper, we report the life history and damage of *T. pegani* and its potential as a biocontrol agent of *P. harmala*.

Material and Methods

Following the discovery of *T. pegani* on *P. harmala*, in Igdir Province in the summer of 2002, we to studied its life history, feeding damage, and parasitoid complex in the field and in the laboratory at Atatürk University from 2003–2005.

Between April and September at about two to three week intervals we made observations in the study area. The plants infested with *T. pegani* were dug up and brought to the laboratory, put in containers with water, and kept in muslin-covered cages (95×45×32 cm) at 24 ± 1°C and approximately 60% RH. Twenty newly hatched larvae were followed to determine the number of larval instars and stadial durations. Infested stems were opened with a razor blade at two-day intervals; the stems were checked until the lavae were ready to pupate. Head capsule widths, larvae lengths, and the lengths and widths of pupae and adults were measured with a Leica MZ 16 FA multi-focusing digital micrometer.

To determine the infestation ratio of *P. harmala* by *T. pegani*, 30 locations were randomly selected and uninfested and infested plants were recorded in 25 m^2 (5×5 m, frame) areas at each location.

Seeds were harvested from uninfested and infested plants at the end of the season and were kept separate for use in 1,000-seed weight, standard germination, and seedling emergence tests.

We counted out 1,000 seeds per sample from collections obtained from both uninfested and infested plants. The experiment was replicated four times per location.

Standard germination tests were conducted in four runs with being four replicates each using 100 seeds for each replicate from uninfested and infested *P. harmala* plants. The seeds were incubated at 23±1°C in 9 cm Petri dishes between two filter paper discs saturated with distilled water containing benlate 1 g/liter to prevent fungal growth. Seeds with visible radical protrusion were considered germinated. Germination was recorded and germinated seeds were discarded at 24 h-intervals for 15 d (ISTA 1996).

For seedling emergence tests, 100 *P. harmala* seeds were sown into 1 cm deep in a loamy soil in rows in four runs with four different emergence tests with three replicates for each group of seeds germination trays at 23±1°C. Emergence percentages of the seeds were calculated for both uninfested and infested plants for 15 d.

To obtain parasitoids, stems with eggs, larvae and pupae were cut into 15 cm sections and kept in plastic containers provisioned with humid sandy soil and covered with muslin. Distilled water was added daily to keep the soil humid. Emerging parasitoids were collected with a mouth-operated aspirator.

To isolate the fungus, samples were taken from both infested and uninfested areas of stem and the body surface of *T. pegani*. Insects were washed under running tap water in a tub, surface disinfected in 1% sodium hypochlorite for 1 min, crushed in sterile Petri dishes, and then placed on 1.5% water agar (WA) and potato dextrose agar (PDA) containing 50 mg/liter streptomycin sulfate.

Laboratory experiments were conducted as randomized complete block designs, with each treatment replicated four times (% 0.05). Results were subjected to ANOVA and the differences between means were compared using a LSD test. A significance level of $P<0.05$ was used for all experiments.

Results

Description.—Adult (Fig. 2d): Newly emerged adult light brown initially but darkens with age. Color variable, from

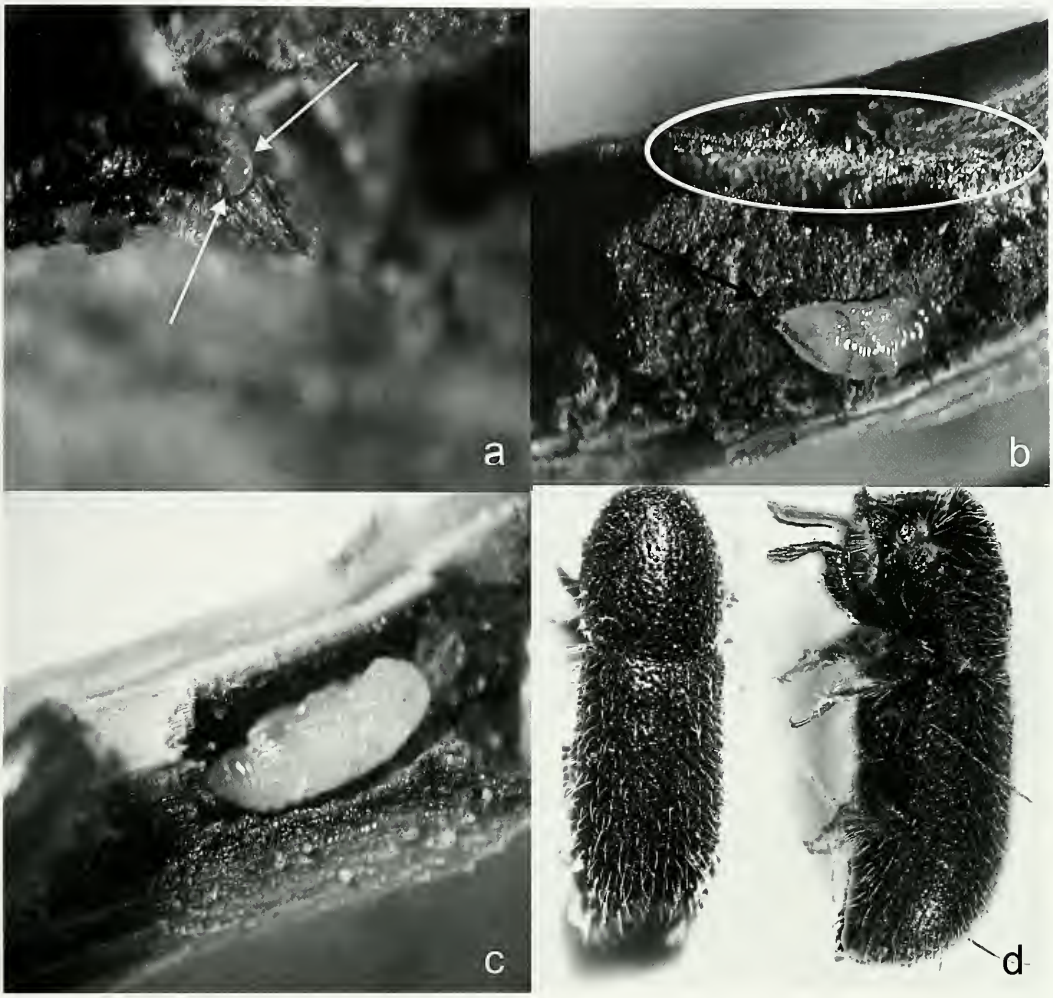

Fig. 2. Stages of *Thamnurgus pegani*. a) Egg, b) Larva in the gallery with *Fusarium oxysporium*, c) Pupa in the gallery, d) Adult.

reddish to blackishbrown. Body elongate and cylindrical, elytra striate, elytral tips truncate. Body covered with golden hairs. Length (mean ± SE) 3.1 ± 0.4 mm (range: 2.8–3.5, n=30) and width 0.9 ± 0.01 mm (range: 0.8–1.0, n=30).

Egg (Fig 2a): Elongate, light yellow, becoming darker with age, and 0.78 ± 0.01 mm (range 0.75–0.81, n=4) long and 0.38 ± 0.01 mm (range 0.35–0.41, n=4) wide.

Larva (Fig. 2b): Mature larva cream colored and elongate. Measurements of first-instar larva (head capsule with body length) 0.27 ± 0.01 (range: 0.24–0.30, n=5), 1.58 ± 0.06 mm (range 1.64–2.79, n=5); second-instar 0.40 ± 0.09 mm (range: 0.35–0.44, n=20), 2.43 ± 0.12 mm (range 1.64–2.79, n=20), and third instar 0.58 ± 0.05 mm (range 0.56–0.61, n= 15), 3.22 ± 0.07 mm (range 2.78–3.51, n= 15). Aksentyev (1991) provided a description of the head capsule.

Pupa (Fig. 2c): Newly emerged pupa cream colored but darkens with age, covered by frass produced by last-instar larva. It measures 4.1 ± 0.1 (range: 3.8–

Fig. 3. a) Distribution of *Peganum harmala* in a meadow, b) *Peganum harmala* infested by *Thamnurgus pegani*, c–d) *Peganum harmala* stems damaged by *Thamnurgus pegani*.

4.5 n=5) mm long and 1.1 ± 0.05 (range:1.1–1.3, n=5) mm wide.

Life history and damage.—From mid-August until early September, some adult beetles leave the galleries and move to the crown of the plant or to the ground, but most adults overwinter in the larval galleries in the damaged stems. Stems harbor from one up to 10 adults, one with as many as 10, but mostly 6 and 7. We also found overwintering adults, with most stems usually containing six to seven. Overwintering beetles appear in the field in late May and move to new stems. In nature, egg-laying started during the last week of May. The female first made a 0.2–0.5 mm diameter cavity in stem with her mouthparts, usually between the stem and lateral branch junction (Fig. 3c) and then inserted an egg. We did not determine the duration of the oviposition period precisely, but we assume that it lasted approximately two weeks. Eggs also are found occasionally in the larval galleries (Fig. 2a). Upon hatching, larvae feed downward along the inside of the stem forming distinctive tunnels. Larvae were first

Table 1. Infestation ratio of *P. harmala* by *T. pegani* in the study area (A: uninfested; B: infested plants).

Year	Mean A/B	Std. Error of Mean A/B	Range A/B	N (Frame)	Sum Plant A/B
2004	4.80/7.46	0.46/0.43	2–11/3–12	30	144/224
2005	5.13/6.53	0.48/051	0–11/1–11	30	154/196

encountered in the galleries at the beginning of June. In July we observed both larvae and pupae in the galleries. Feeding tunnels were 5.24 ± 0.45 mm (range: 5–7 n=5) mm long and 1.5 ± 0.83 mm. (range: 1.4–1.6, n=5) mm wide. In one stem, including all associated side shoots, we counted six tunnels. The color of the feeding tunnel becomes blackish brown due to the presence of the fungus (Fig. 3d). This damage caused plants to lose their normal texture, become dry and fragile, and break easily. Additionally, fruits from infested plants were smaller than those from uninfested plants.

The fungus, *Fusarium oxysporium*, was detected within the larval galleries and on the body of *T. pegani*. This fungus infests the plant tissue and propagates within the galleries and within the stem both above and below the larval galleries (Fig. 2b). Fungal-infested tissue becomes blackish brown. The white mycelia of the fungus on the surface of the blackened tissue are highly visible (Fig. 2b, in circle). We believe that the larvae and adults utilize this infected tissue as a food source. We suspect that since this fungus is a soil inhabitant for a portion of its life cycle, overwintering beetles in the soil bear spores in or on their bodies, and when they make feeding or breeding cavities in the stems, inoculate the stems with the fungus. The other possibility, since beetles feed upon fungal-infected tissue, is that spores may adhere to their bodies and overwintering insects inoculate the stem with the pathogen as a consequence of their feeding activities the following spring. Full-grown larvae pupate in the feeding tunnels. New adults appear during the first week of August and they continue feeding in the larval tunnels until leaving for hibernation. *Thamnurgus pegani* completes only one generation a year.

Under laboratory conditions, the egg incubation period required 9.06 ± 1.57 d (range: 7–11, n=15). The duration of the first larval instar was 8.13 ± 0.83 d (range: 7–9 n=15); second instar 11.85 ± 0.81 d (range 11–13, n=20); and the third instar was 10.05 ± 0.94 d (range7–8, n=20) days. The pupal period lasted 8.46 ± 0.15 d (range: 8–9, n=15).

In the study area in 2004, the number (mean ± SE) of *P. harmala* plants attacked by *T. pegani* was 7.46–0.43 (range 3–12, n=30 frames) and unattacked plants 4.8 ± 0.43 (range 2–11, n=30 frames). The total number of plants sampled was 368 (Table 1). Differences between means of infested versus uninfested plants were statistically significant ($P<0.05$, df: 5, F: 17.56). In 2005, the mean number of infested plants was 6.53 ± 0.51 (range 1–11 n=30 frames) and uninfested plants 5.13± 0.48 (range 0–11 n=30 frames). The total number of plants sampled was 350 (Table 1). These values were statistically significant ($P<0.05$, df: 7, F: 3.8).

The mean weight of 1,000 seeds obtained from uninfested and infested plants was 1.95± 0.29 (range 1.86–2.00, n=4), from uninfested plants and 1.57±0.03 g (range 1.50–1.68, n=4) from infested plants. The difference between means was statistically significant ($P<0.05$, df: 1, F: 63.39).

The germination started to on fourth day and terminated on tenth day

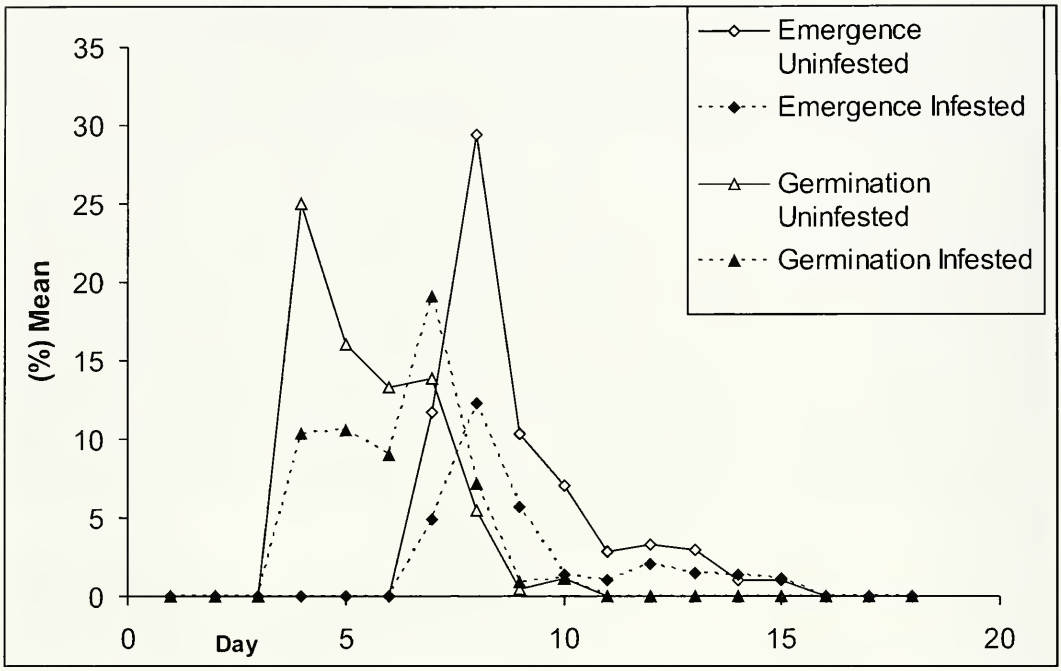

Fig. 4. Germination and emergence of seeds obtained from uninfested and infested plants.

(Fig. 4). Daily mean germinating percentage from infested plants was 8.31 ± 1.30 (%). Daily mean germination percentage from uninfested plants was 10.73 ± 1.84 (Table 2). The difference between seed germination percentage for uninfested and infested plants were statistically significant ($P<0.05$, df: 1, F: 4.1). However, among tests and replications, germination percentage were not significant ($P>0.05$, df: 9, F: 0.20).

The emergence started to on seventh day and terminated on fifteenth day (Fig. 4). Daily mean seedlings emergence of seeds obtained from beetle-infested plants was 3.48 ± 0.73 (%) (Table 2). Daily emergence of seedlings ratio emergence of seeds from uninfested plants was 7.75 ± 1.83 (%) (Table 2). The difference seedling emergence percentage between infested and uninfested plants were significant ($P<0.05$, df: 1, F: 17.08). There is no difference between trials and replications ($P>0.05$, df: 6, F: 0.75).

Parasitoids.—No parasitoids were found during field collections and labo-

Table 2. % Germination and emergence of seeds obtained from uninfested and infested plants.

		Mean (%) ± Std. Error	Germination from 1600 Seed	Total Germination (%)
Germination (%)	Uninfested	10.73* ± 1.84	1202	75.11
	Infested	8.31* ± 1.30	931	58.17
		Mean (%) ± Std. Error	**Emergence from 1200 Seed**	**Total Emergence (%)**
Emergence (%)	Uninfested	7.75[q] ± 1.83	836	69.75
	Infested	3.48[q] ± 0.73	276	31.32

* Daily mean germinating percentage; [q] Daily mean emergence percentage.

ratory rearing of eggs, pupae, and adults. In the laboratory, the parasitoids *Coeloides foersteri* Haes (Hymenoptera: Braconidae,), and *Homoporus* sp. and *Trichomalus* sp. (Hymenoptera: Pteromalidae) were obtained from the larvae. *Coeloides foersteri* is an idiobiont parasitoid.

Conclusion

In this study, the scolytid, *Thamnurgus pegani* was first detected in Igdır Province in the foothills of Mt. Ararat feeding on *Peganum harmala*, a common and abundant plant especially in the eastern and central part of Turkey and toxic to domestic animals. We also observed the occurrence of this insect in other areas of eastern Turkey.

Thamnurgus pegani is a stem feeder, the adults and larvae feed in the stem forming galleries within the pith of the host. It is notable that the fungus, *Fusarium oxysporum*, develops within these tunnels. Insect feeding and fungal attack weakens the plants. It is remarkable to emphasize that presence of the fungus in stem could increase the biocontrol effect of the beetle. Additionally, the color of the leaves close to the infected area of the stem turns yellow and dry. Fruits from infested plants are smaller than those from uninfested plants. The differences of mean thousand seed weight, standard germination tests, and trial tests of seeds obtained from uninfested and infested plants results from the combined insect and fungus occurrence. *Thamnurgus pegani* is a monophagous species, known only to attack *P.harmala*. In areas occupied by *P.harmala*, *Euphorbia virgata* Woldst et Kit. is abundantly present, but we did not observe *T. pegani* feeding injury to this plant species even though *Thamnurgus* spp. are known to use *Euphorbia* spp. as hosts. *Thamnurgus. pegani* is a potential candidate for the biological control of *P.harmala*, but further research is required to fully assess its role.

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